Obesity, Blood Pressure, and the Sympathetic Nervous System

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Obesity has long been recognized as a major risk factor for the development of hypertension. Recently, insulin level has been shown to correlate with blood pressure in clinical and population-based studies. Since insulin is a major signal in the relationship between dietary intake and sympathetic nervous system activity, the possibility that insulin-mediated sympathetic stimulation is involved in the pathogenesis of hypertension in the obese has been raised. This hypothesis, developed on the basis of studies in laboratory rodents and normal human subjects, is currently being tested in the Normative Aging Study in Boston. Utilizing epidemiologic techniques applied to this defined population, evidence in support of this hypothesis has been accumulated. The preliminary results indicate that in this population, the abdominal form of obesity is associated with higher insulin levels and increased 24-hour urinary norepinephrine excretion (an index of sympathetic activity). Ann Epidemiol 1991;1:295–303

KEY WORDS: *Insulin, sympathetic nervous system, obesity, diet, blood pressure.*

INTRODUCTION

The association of obesity and hypertension has been solidly established (1–6). Hypertension, moreover, contributes substantially to the cardiovascular risk associated with the obese state (1, 7, 8). The nature of the relationship between obesity and hypertension, however, remains obscure. Recent studies demonstrating a relationship between body fat distribution, insulin resistance and hyperinsulinemia, and blood pressure (9–20) suggested the hypothesis depicted in Figure 1. This hypothesis may be summarized as follows: (1) Obesity, principally in its centripetal or abdominal form, is associated with insulin resistance and hyperinsulinemia; (2) hyperinsulinemia stimulates the sympathetic nervous system (SNS), driving sympathetically mediated thermogenesis and thereby increasing energy expenditure; and (3) the effects of insulin to stimulate renal sodium reabsorption, and the effects of sympathetic stimulation of the kidneys, blood vessels, and heart, result in hypertension. According to this hypothesis, insulin resistance and hyperinsulinemia are mechanisms recruited in the obese to limit further weight gain by restoring energy balance. In physiologic terms, the price exacted for this compensatory mechanism is chronic hyperinsulinemia and SNS stimulation, both of which might contribute to the development of hypertension (21). Central to this hypothesis is the relationship between dietary intake and SNS activity.

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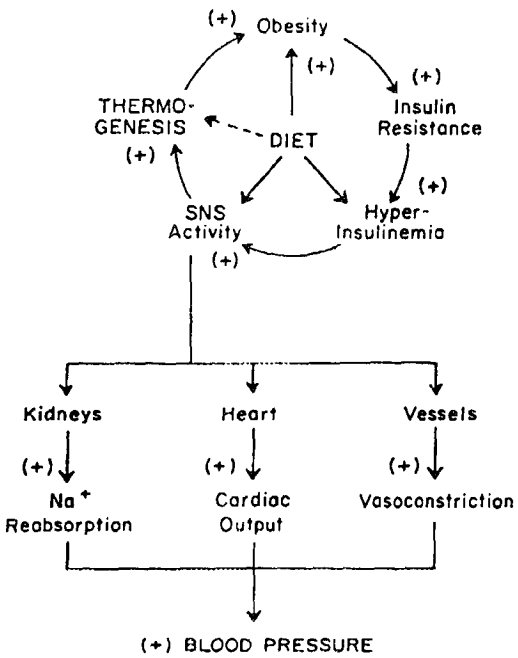

FIGURE 1 Relationship between obesity, insulin, sympathetic nervous system (SNS) activity, thermogenesis, and blood pressure. See text for details. (Reprinted with permission of Oxford University Press from Landsberg L, in *Quarterly Journal of Medicine* 1986; 61:1081–1090.)

Diet and SNS Activity

Dietary intake profoundly affects sympathetic activity: Fasting and caloric restriction suppresses, while overfeeding stimulates the SNS (22–25). Sympathetic responses to dietary intake are, moreover, nutrient specific; carbohydrate and fat stimulate the SNS, even when total caloric intake is not increased (26–28) while protein is without effect (29). Although originally described in laboratory rodents, it is clear that diet influences sympathetic activity in humans as well (30, 31).

Role of Insulin

Evidence has accumulated that insulin-mediated glucose metabolism in neurons related to the ventromedial hypothalamus play a critical role in the relationship between diet and sympathetic activity (32). A signaling mechanism between the periphery and the central nervous system is obviously required since sympathetic outflow is regulated at the level of the hypothalamus and brainstem. Initial clues implicating glucose in this relationship were derived from studies of experimental hypoglycemia; decreased blood glucose levels in different circumstances (33, 34) diminished SNS activity despite the concomitant stimulation of the adrenal medulla. The importance of glucose metabolism was demonstrated by studies involving 2-deoxy-D-glucose. This agent, which diminishes intracellular glucose metabolism, suppressed sympathetic activity despite an increase in blood glucose level and an increase in dietary intake (35). A role for insulin in this relationship was demonstrated by the fact that experimental diabetes diminished sympathetic activity despite hyperglycemia and hyperphagia (36) and that insulin

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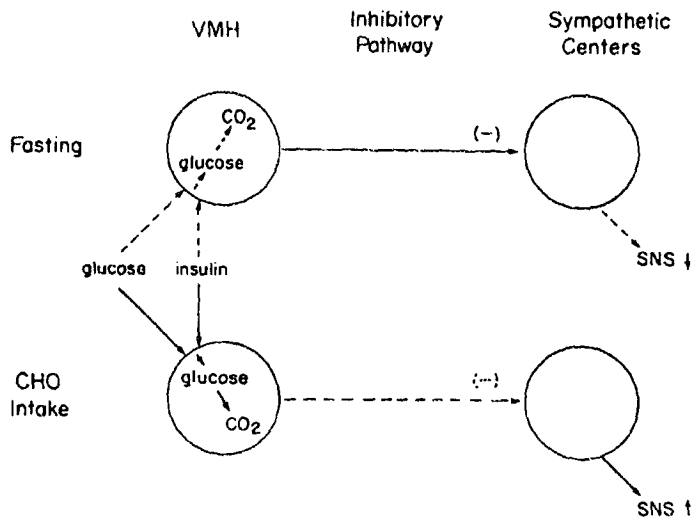

FIGURE 2 Insulin-mediated glucose metabolism in the regulation of sympathetic nervous system (SNS) activity. During fasting, diminished glucose and insulin levels decrease intracellular glucose metabolism in critical central neurons related to the ventromedial hypothalamus (VMH). This decrease in glucose metabolism stimulates an inhibitory pathway, resulting in suppressed sympathetic outflow. Conversely, during carbohydrate intake or in the presence of insulin resistance, in which both glucose and insulin levels are increased, insulin-mediated glucose metabolism in these same cells is increased. This results in suppression of the inhibitory pathway with a resultant increase in sympathetic outflow from tonically active lower centers in the brainstem. (Reprinted with permission of W. B. Saunders from *Clinics in Endocrinology and Metabolism* 1984; 13:475–499.)

ous system (SNS) activity, with permission of Oxford 86; 61:1081–1090.)

administration, with sufficient carbohydrate to avoid hypoglycemia, stimulated the SNS in both rats (37) and humans (38). Ablation of insulin-glucose–sensitive neurons with gold thioglucose in mice, furthermore, completely obliterated diet-induced changes in SNS activity (39). These relationships are summarized in Figure 2.

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Physiologic Significance of Dietary Changes in SNS Activity

It is reasonably well established that diet-induced changes in sympathetic activity contribute importantly to the phenomenon known as dietary thermogenesis (40–43). This term refers to alterations in metabolic rate induced by changes in nutritional status, and includes the effect of antecedent diet on the resting metabolic rate. Suppression of sympathetically mediated thermogenesis by fasting or caloric restriction may have evolved as a mechanism to conserve calories during periods of famine. The origins of increased sympathetically mediated thermogenesis in response to carbohydrate and fat intake are less clear but may be related to the need to secure adequate supplies of nitrogen in the face of protein-deficient diets (28, 44). By overeating a subsistence diet low in protein, an organism would be able to satisfy basic nitrogen requirements for growth and development while dissipating the excess calories and avoiding obesity. Whatever the evolutionary origins, it should be clear that the capacity for sympathetically mediated thermogenesis conveys resistance to weight gain by increasing metabolic rate and hence energy expenditure. The hypothesis developed here predicts that the hyperinsulinemia of obesity drives the SNS and increases metabolic rate (see Figure 1) as a mechanism recruited to limit further weight gain and

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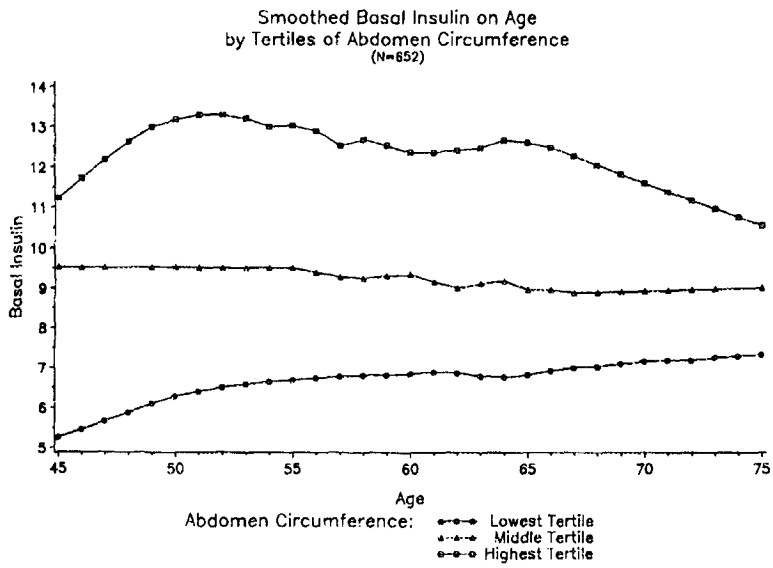

FIGURE 3 "Smoothed" basal insulin levels as a function of age plotted by tertiles of abdominal circumference. Basal insulin is in μIU/mL. Note that the lowest insulin levels occur in those with the smallest abdominal girth, whereas the highest fasting insulin levels are noted in those with the greatest abdominal circumference.

stabilize body weight. The portion of this hypothesis that predicts increased insulin-mediated sympathetic stimulation in subjects with abdominal obesity is being tested in the population-based Normative Aging Study in Boston. Preliminary results of these studies are reported here.

MATERIALS AND METHODS

The Normative Aging Study has been previously described in detail (45). Subjects involved in this study report for examinations every 3 to 5 years. In addition to the ongoing observations that include a physical and anthropometric examination and blood and urine tests, participating subjects provided a 24-hour urine collection that was analyzed for catecholamines, and blood for insulin determinations in the fasting state. Urinary norepinephrine and epinephrine levels were determined on the 24-hour urine sample utilizing high-performance liquid chromatography (HPLC) with electrochemical detection (46, 47). The intra-assay coefficient of variation for the urine samples was 4 to 6% and the interassay coefficient of variation was 6 to 7%. Results were corrected for recovery by an internal standard. Insulin was determined by a solid-phase radioimmunoassay in the GCRC Core Laboratory of the Beth Israel Hospital (Coat-A-Count insulin 1987; Diagnostic Products Corporation, Los Angeles, CA). Abdomen circumference was measured to the nearest 1.0 cm at the level of the umbilicus, perpendicular to the axis of the body. All measurements were made with the subjects in undershorts and socks, standing erect with feet together.

The 24-hour urine samples were collected at home by the participants and brought to the Normative Aging Study at the time of examination. All participating subjects completed a questionnaire eliciting information on the urine collection, including

RESULTS

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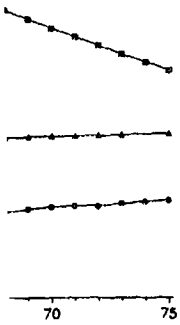

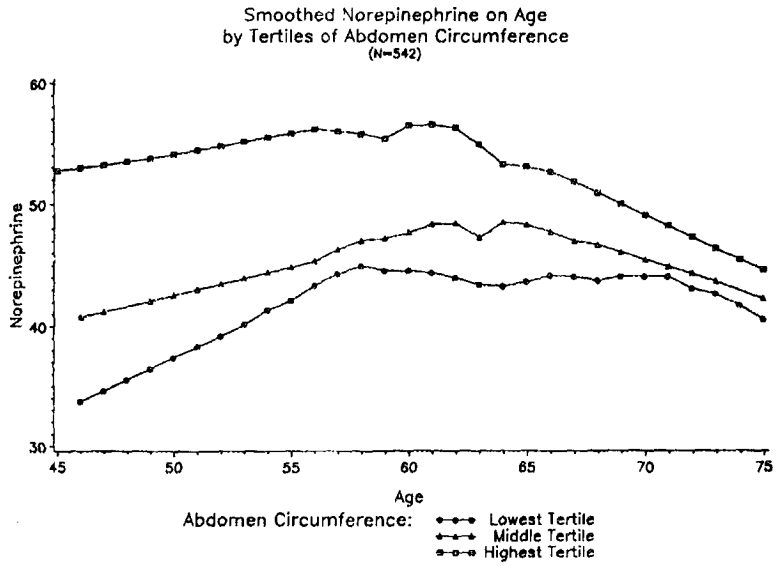

FIGURE 4 "Smoothed" norepinephrine excretion levels as a function of age by tertiles of abdominal circumference. Norepinephrine is represented as μg/24 h. Note that the subjects with the greatest abdominal girth excreted the most norepinephrine. The pattern resembles that shown for insulin in Figure 3.

times of collection, miscollections, spilled urine, and medication use. Data were collected from examinations conducted between February 1987 and May 1989. Eighty-one percent of the subjects provided a 24-hour urine sample. After exclusions on the basis of inadequate urine volumes or collection times, medication use (L-dopa, methyl dopa, or insulins), or missing values for one or more of the study variables, 542 observations of catecholamine excretion were available for the present evaluation. Six hundred fifty-two subjects were included in the analysis involving fasting insulin level.

The data are presented as basal insulin (μlU/mL) or 24-hour catecholamine excretion (μg/24 h) as a function of age for the lowest, middle, and highest tertile of abdominal circumference of the study population.

Smoothed curves were estimated for basal insulin and 24-hour urinary norepinephrine and epinephrine levels by tertile of abdomen circumference versus age, using the methods and algorithm proposed by Friedman and Stuetzle (48). This nonparametric graphic curve estimation technique estimates a value for urinary norepinephrine at any age by fitting a least-squares straight line to all the urinary norepinephrine values within a given abdomen circumference tertile at a particular age. Although linear in the "neighborhood" of a particular age value, the technique both is sensitive to the amount of data at any age and accommodates changes in slope of the least-squares estimator such that the overall curve is not constrained to be linear and reflects the mass of the data at any age value (49).

RESULTS

The relationship between basal insulin levels and abdominal circumference as a function of age is shown in Figure 3. Throughout the entire age range of the population, subjects with the greatest abdominal girth had the highest fasting insulin levels, while

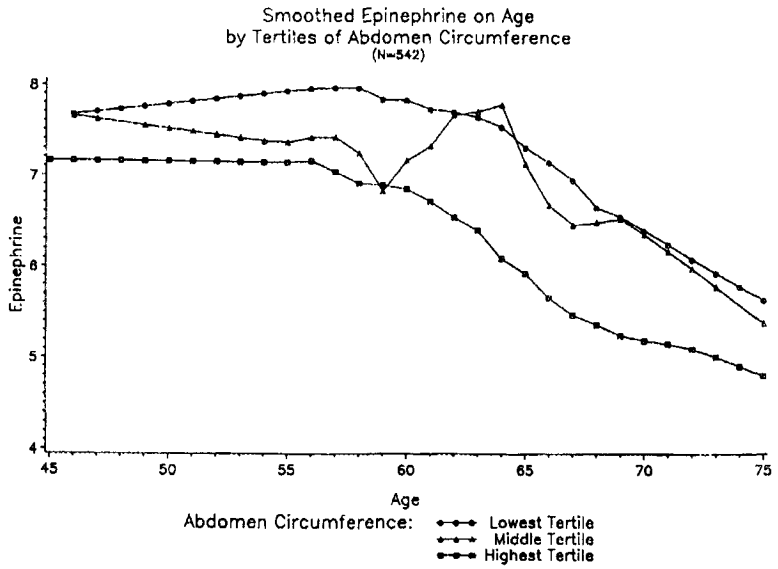

FIGURE 5 "Smoothed" epinephrine excretion levels as a function of age by tertiles of abdominal circumference. Epinephrine excretion is represented as $\mu g/24$ h. Note the inverse relationship between abdominal girth and epinephrine excretion, a pattern opposite that demonstrated for norepinephrine shown in Figure 4. Note also the decrease in epineprhine excretion with age.

those with the lowest abdominal girth had the lowest levels of insulin. The relationship between norepinephrine excretion and abdominal circumference as a function of age is shown in Figure 4. The pattern resembles that noted for a fasting insulin although the differences among the tertiles of abdominal circumference are most marked in individuals below the age of 65. The difference is also greatest between the middle and the highest tertile of abdominal circumference. Epinephrine secretion, on the other hand, follows a different pattern (Figure 5). Abdominal girth and epinephrine excretion are inversely related; those individuals in the highest tertile of abdominal circumference have lower 24-hour urinary excretion levels of epinephrine. Epinephrine excretion also tends to diminish after the age of 60 years. Body mass index (data not shown) has a similar relationship to catecholamine excretion as does abdominal circumference. These nonparametric smoothed curves do not provide a measure of variability by which we can calculate statistical significance of the tertile differences reported here. Data analysis with conventional parametric techniques does allow assessment of the statistical significance of these differences. Regression analysis indicates a significant direct relationship between insulin level and abdominal circumference, and urinary norepinephrine excretion and abdominal circumference. An inverse relationship between abdominal circumference and epinephrine excretion is also significant.

DISCUSSION

The relationship between abdominal girth and fasting insulin levels demonstrated here for the Normative Aging Study population is consistent with a large body of evidence linking the upper body, abdominal form of obesity to hyperinsulinemia and insulin resis-

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tance (9, 16, 50). The relationship between abdominal girth and urinary norepinephrine excretion, on the other hand, has not been previously reported. Since urinary norepinephrine excretion provides an integrated measure of plasma norepinephrine levels over time, enriched by sympathetic activity directed at the kidney (51), these results are consistent with increased sympathetic activity in the obese. This observation is of interest since the relationship between obesity and SNS activity is an area of continuing controversy. Since some genetic rodent models of obesity are associated with diminished sympathetic activity (52–55), an "autonomic hypothesis" has been developed in which diminished sympathetic activity is hypothesized to contribute to the development of obesity by impairing sympathetically mediated thermogenesis, thereby increasing metabolic efficiency. Studies in humans produced evidence of diminished, unchanged, or increased SNS activity (56). In the group of obese subjects studied here, the abdominal form of obesity appears to be associated with enhanced SNS activity.

The parallelism between fasting insulin levels and urinary norepinephrine excretion demonstrated in Figures 3 and 4 is consistent with, but does not prove, that insulin is involved in sympathetic stimulation, as suggested in the hypothesis outlined in Figure 1. Further studies are necessary to demonstrate the linkage between insulin and the SNS. The different pattern demonstrated for epinephrine excretion (see Figure 5) enhances the specificity of the changes demonstrated with norepinephrine. The inverse relationship to abdominal girth makes it unlikely that nonspecific effects related to body size influenced catecholamine excretion since, under these circumstances, norepinephrine and epinephrine should behave similarly. Suppression of epinephrine with elevated insulin and glucose levels has been shown to occur in clinical studies (57), an observation reproduced in this population-based study. These observations also provide further evidence of dissociation between SNS and adrenal medullary responses, a dissociation that was demonstrated previously in experimental animals (31).

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REFERENCES

1. Van Itallie TB. Health implications of overweight and obesity in the United States, Ann Intern Med. 1985;103:983–8.

2. Havlik RJ, Hubert HB, Fabsitz RR, Feinleib M. Weight and hypertension, Ann Intern Med. 1983;98:855–9.

3. Chiang BN, Perlman LV, Epstein FH. Overweight and hypertension, Circulation. 1969;39:403–21.

4. Berchtold P, Jorgens V, Finke C, Berger M. Epidemiology of obesity and hypertension, Int J Obesity. 1981;5:1–7.

5. Kannel WB, Brand N, Skinner JJ Jr, Dawber TR, McNamara PM. The relation of adiposity to blood pressure and development of hypertension, Ann Intern Med. 1967;67:48–59.

6. Gordon T, Kannel WB. Obesity and cardiovascular disease: The Framingham Study, J Clin Endocrinol Metab. 1976;5:367–75.

7. Van Itallie TB, Abraham S. Some hazards of obesity and its complications. In: Hirsch J, Van Itallie TB, eds. Recent Advances in Obesity Research. V. IV. London: John Libbey; 1985:1–19.

8. Grundy SM, Greenland P, Herd A, et al. Cardiovascular and risk factor evaluation of healthy American adults, Circulation. 1987;75:1340A–62A.

9. Kalkhoff RK, Hartz AH, Rupley D, Kissebah AH, Kelber S. Relationship of body fat distribution to blood pressure, carbohydrate tolerance, and plasma lipids in healthy obese women, J Lab Clin Med. 1983;102:621–7.

10. Weinsir RL, Norris DJ, Birch R, et al. The relative contribution of body fat pattern to blood pressure level, Hypertension. 1985;7:578–85.

11. Gillum RF. The association of body fat distribution with hypertension, hypertensive heart disease, coronary heart disease, diabetes and cardiovascular risk factors in men and women aged 18–79 years, J Chronic Dis. 1987;40:421–8.

12. Seidell JC, Bjorntorp P, Sjostrom L, Sannerstedt R, Krotkiewski M, Kvist H. Regional distribution of muscle and fat mass in men: New insight into the risk of abdominal obesity using computed tomography, Int J Obesity. 1989;13:289–303.

13. Selby JV, Friedman GD, Quesenberry CP Jr. Precursors of essential hypertension: The role of body fat distribution pattern. Am J Epidemiol 1989;129:43–53.

14. Peiris AN, Sothmann MS, Hoffmann RG, et al. Adiposity, fat distribution, and cardiovascular risk, Ann Intern Med. 1989;110:867–72.

15. Krieger DR, Landsberg L. Mechanisms in obesity-related hypertension: Role of insulin and catecholamines, Am J Hypertens. 1988;1:84–90.

16. Peiris AN, Mueller RA, Smith GA, Struve MF, Kissebah AH. Splanchnic insulin metabolism in obesity, J Clin Invest. 1986;78:1648–57.

17. Rose HG, Yalow RS, Schweitzer P, Schwartz E. Insulin as a potential factor influencing blood pressure in amputees, Hypertension. 1986;8:793–800.

18. Manicardi V, Camellini L, Bellodi G, Coscelli C, Ferrannini E. Evidence for an association of high blood pressure and hyperinsulinemia in obese man, J Clin Endocrinol Metab. 1986;62:1302–4.

19. Modan M, Halkin H, Almog S, et al. Hyperinsulinemia: A link between hypertension, obesity and glucose intolerance, J Clin Invest. 1985;75:809–17.

20. Ferrannini E, Buzzigoli G, Bonadonna R, et al. Insulin resistance in essential hypertension, N Engl J Med. 1987;317:350–6.

21. Landsberg L. Diet, obesity and hypertension: An hypothesis involving insulin, the sympathetic nervous system, and adaptive thermogenesis, Q J Med. 1986;236:1081–90.

22. Young JB, Landsberg L. Suppression of sympathetic nervous system during fasting, Science. 1977;196:1473–5.

23. Young JB, Landsberg L. Stimulation of the sympathetic nervous system during sucrose feeding, Nature. 1977;269:615–7.

24. Young JB, Saville E, Rothwell NJ, Stock MJ, Landsberg L. Effect of diet and cold exposure on norepinephrine turnover in brown adipose tissue in the rat, J Clin Invest. 1982;69:1061–71.

25. Landsberg L, Young JB. The influence of diet on the sympathetic nervous system. In: Muller EE, MacLeod RM, Frohman LA, eds. Neuroendocrine Perspectives. Amsterdam: Elsevier Science; 1985:191–218.

26. Walgren MC, Kaufman LN, Young JB, Landsberg L. The effects of various carbohydrates on sympathetic nervous system activity in heart and interscapular brown adipose tissue (IBAT) of the rat, Metabolism. 1987;36:585–94.

27. Schwartz JH, Young JB, Landsberg L. Effect of dietary fat on sympathetic nervous system activity in the rat, J Clin Invest. 1983;72:361–370.

28. Young JB, Kaufman LN, Saville ME, Landsberg L. Increased sympathetic nervous system activity in rats fed a low-protein diet, Am J Physiol. 1985;248:R627–37.

29. Kaufman LN, Young JB, Landsberg L. Effect of protein on sympathetic nervous system activity in the rat: Evidence for nutrient-specific responses, J Clin Invest. 1986;77:551–8.

30. O'Dea K, Esler M, Leonard P, Stockigt JR, Nestel P. Noradrenaline turnover during under- and over-eating in normal weight subjects, Metabolism. 1982;31:896–9.

31. Young JB, Rosa RM, Landsberg L. Dissociation of sympathetic nervous system and adrenal medullary responses, Am J Physiol. 1984;247:E35–E40.

32. Landsberg L, Young JB. Insulin-mediated glucose metabolism in the relationship between dietary intake and sympathetic nervous system activity, Int J Obesity. 1985;9:63–8.

33. Young JB, Landsberg L. Sympathoadrenal activity in fasting pregnant rats: Dissociation of adrenal medullary and sympathetic nervous system responses, J Clin Invest. 1979;64:109–16.

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34. Landsberg L, Greff L, Gunn S, Young JB. Adrenergic mechanisms in the metabolic adaptation to fasting and feeding: Effects of phlorizin on diet-induced changes in sympathoadrenal activity in the rat, Metabolism. 1980;29:1128–37.

35. Rappaport EB, Young JB, Landsberg L. Effects of 2-deoxy-D-glucose on the cardiac sympathetic nerves and the adrenal medulla in the rat: Further evidence for a dissociation of sympathetic nervous system and adrenal medullary responses, Endocrinology. 1982;110:650–6.

36. Young JB, Einhorn D, Landsberg L. Decreased sympathtic (SNS) activity in interscapular brown adipose tissue (IBAT) of streptozotocin-treated rats, Diabetes. 1983;32(suppl):26A.

37. Young JB. Effect of experimental hyperinsulinemia on sympathetic nervous system activity in rat, Life Sci. 1988;49:193–200.

38. Rowe JW, Young JB, Minaker KL, Stevens AL, Pallotta J, Landsberg L. Effect of insulin and glucose infusions on sympathetic nervous system activity in normal man, Diabetes. 1981;30:219–25.

39. Young JB, Landsberg L. Impaired suppression of sympathetic activity during fasting in the gold thioglucose-treated mouse, J Clin Invest. 1980;65:1086–94.

40. Acheson K, Jequier E, Wahren J. Influence of beta-adrenergic blockade on glucose-induced thermogenesis in man, J Clin Invest. 1983;72:981–6.

41. Acheson KJ, Ravussin E, Wahren J, Jequier E. Thermic effect of glucose in man, J Clin Invest. 1984;74:1572–80.

42. Jung RT, Shetty PS, James WPT. The effect of beta-adrenergic blockade on metabolic rate and peripheral thyroid metabolism in obesity, Eur J Clin Invest. 1980;10:179–82.

43. Landsberg L, Young JB. The role of the sympathoadrenal system in modulating energy expenditure, J Clin Endocrinol Metab. 1984;13:475–99.

44. Landsberg L. Obesity, metabolism and hypertension, Yale J Biol Med. 1989;62:511–9.

45. Bell B, Rose CL, Damon H. The Normative Aging Study: An interdisciplinary and longitudinal study of health and aging, Aging Hum Dev. 1972;3:5–17.

46. Smedes F, Kraak JC, Poppe H. Simple and fast solvent extraction system for selective and quantitative isolation of adrenaline, noradrenaline and dopamine from plasma and urine, J Chromatogr. 1982;231:25–39.

47. MacDonald IA, Lake DM. An improved technique for extracting catecholamines from body fluids, J Neurosci Methods. 1985;13:239–48.

48. Friedman JH, Stuetzle W. Smoothing of Scatterplots. Monograph 003, Department of Statistics. Stanford, CA: Stanford University; 1982.

49. Segal MR, Tager IB, Weiss ST, Speizer FE. Smoothing methods for epidemiologic studies, Stat Med. 1988;7:601–11.

50. Smith U. Regional differences in adipocyte metabolism and possible consequences in vivo. In: Hirsch J, Van Itallie TB, eds. Recent Advances in Obesity Research IV, Proceedings of the 4th International Congress on Obesity. London: John Libbey; 1983:33–6.

51. Landsberg L, Young JB. Catecholamines and the adrenal medulla. In: Foster DW, Wilson JD, eds. Williams Textbook of Endocrinology. Philadelphia: WB Saunders; 1985:891–965.

52. Young JB, Landsberg L. Diminished sympathetic nervous system activity in the genetically obese mouse, Am J Physiol. 1983;245:E148–54.

53. Bray GA, York DA. Hypothalamic and genetic obesity in experimental animals: An autonomic and endocrine hypothesis, Physiol Rev. 1979;59(3):719–809.

54. Fisler JS, Bray GA. Dietary obesity: A metabolic hypothesis. In: Bray GA, Ricquier D, Spiegelman BM, eds. Obesity: Towards a Molecular Approach. New York: Wiley-Liss; 1990:29–43.

55. Landsberg L, Young JB. Obesity and the sympathetic nervous system. In: Bray GA, Ricquier D, Spiegelman BM, eds. Obesity: Towards a Molecular Approach. New York: Wiley-Liss; 1990:81–93.

56. Kush RD, Young JB, Katzeff HL, et al. Effect of diet on energy expenditure and plasma norepinephrine in lean and obese Pima Indians, Metabolism. 1986;35:1110–20.

57. Schwartz RS, Ravussin E, Massari M, O'Connell M, Robbins DC. The thermic effect of carbohydrate versus fat feeding in man, Metabolism. 1985;34:285–93.